IMMUNITY TO HOMOLOGOUS GRAFTED SKIN. III. THE FATE OF SKIN HOMOGRAFTS TRANSPLANTED TO THE BRAIN, TO SUBCUTANEOUS TISSUE, AND TO THE ANTERIOR CHAMBER OF THE EYE.

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SKIN autografts have long been known to survive heterotopic transplantation to almost any part of the body : brain, liver and spleen are as hospitable to skin grafts as skin itself. By grafting skin to regions of the body with major anatomical peculiarities—notably to the brain, which lacks a lymphatic drainage system, and to the anterior chamber of the eye, in which, as *in vitro*, grafts may be kept alive without penetration by blood vessels—the part played by otherwise inaccessible variables in transplantation immunity can be singled out and appraised. The use of heterotopic transplantation for such a purpose is far from new ; but earlier workers have sometimes failed to provide distinct answers to two quite distinct questions. Of skin homografts transplanted to the brain we may, for example, ask :

(*a*) Do such grafts survive transplantation to the brains of non-immunized animals ?

(*b*) Do they survive transplantation to the brains of animals which have been specifically immunized beforehand by graftings of tissue from the same donors to positions of known antigenic effectiveness elsewhere ?

It has been repeatedly shown—first by Shirai (1921) and Murphy (1926), and most recently by Tansley (1946)—that foreign homologous tissues grafted to the brain either do not provoke an immunity reaction or, if they do, do not respond to it. It will be shown here that they *do* respond to an immune state of proved effectiveness called forth by a preliminary grafting of foreign tissue elsewhere. The two questions, relating as they do to quite distinct immunological properties, may thus have contrary answers.

Earlier work gives a less decisive answer to question (*a*) when the site of transplantation is the anterior chamber of the eye. Greene (1940, 1942, 1943) has shown that even heterografts (notably of embryonic and tumour tissue) enjoy a relatively prolonged survival in the anterior chamber of the eye, and found that the anterior chamber of the other eye (or the testicle) became in due course resistant to further inoculations. Cheever and Morgan (1942), reporting on the use of the anterior chamber as a successful culture medium for the Brown-Pearce carcinoma, found that grafting to one eye did not prejudice the success of grafts later transplanted to the other. In experiments of the converse type, Saphir, Appel and Strauss (1941), confirming the observations of Besredka and Bardach (1936), showed that intra-testicular grafts of the Brown-Pearce carcinoma inhibited or altogether suppressed the growth of grafts later transplanted to the anterior

chamber. Apart from the inconsistency mentioned above, grafts to the anterior chamber seem thus to resemble grafts to the brain : they respond to, but do not elicit, an immune state. In this paper it will be shown with strict controls for each experiment that a skin homograft survives transplantation to the eye of a specifically and strongly immunized rabbit if, and only if, it remains unvascularized.

In addition, the experiments to be reported on below provide further evidence for the general rule (Medawar, 1945) that transplantation immunity is the outcome of a systemic and not a local reaction ; and they offer a rational explanation for the well-known clinical success of corneal homografts in human beings.

METHODS.

Full-grown rabbits weighing 2–2½ kg. from dealers' mixed stocks were used throughout. Save where some variant of the standard procedure is specifically mentioned, each individual test has made use of an independent pair of rabbits : a donor (*D*) and a recipient (*R*). (In one of the two groups of eye-implantation experiments, most of the recipients were linked together in pairs by sharing a donor between them. In these particular experiments the variable under investigation was the degree of vascularization of the grafts. As this is a function of operation technique and post-operative history, and not of the immunological relationship between donor and recipient, the procedure is unobjectionable.) Each recipient was immunized by the standard operation (Medawar, 1944, 1945) in which 8 large pinch grafts, each weighing modally 0·045–0·055 g., were cut from the thigh of *D* and accurately fitted to separate raw areas of the appropriate size on the skin overlying left side of the chest of *R*. Fifteen to 20 days after this heavy preliminary immunization, single further skin grafts from *D* were transplanted (*a*) to the centre of a 2 cm. × 2 cm. raw area stripped above the level of the panniculus carnosus from the skin overlying the right side of the chest : and (*b*) to one of the following positions : the left cerebral hemisphere : the sub-integumentary tissue between the panniculus carnosus and the body wall in the chest region ; and the anterior chamber of one eye.

In one group of experiments six days, and in the remainder ten days, was allowed to pass between this second operation and autopsy. The grafts were then examined *in situ* and fixed in formol-$HgCl_2$ for sectioning. The behaviour of the " orthotopic " control graft on the chest (i.e. the skin graft in skin position) provided a standard to which the behaviour of the others could be individually referred. In the majority of cases its long-standing necrosis stood witness to the completeness of immunization. But in some members of the six-day autopsy group (the time interval being chosen for this reason) the control graft showed some degree of partial survival. These experiments made possible a particularly accurate point-for-point comparison between the degree of survival of the control grafts and that of their contemporaries in anatomically unnatural positions elsewhere.

The control grafts, and those transplanted to the brain and below the integument, were rectangles ranging from 2 mm. × 3 mm. to 2½ mm. × 4 mm. in lengths of side, and so cut with a straight-edged scalpel blade as to include with the epidermis only the upper part of the compact connective tissue of the corium.

The best results from eye-implantation grafts were given by the use of squares of only 1 mm. in length of side, though larger grafts (2 mm. × 2 mm.) were used in earlier experiments not specifically reported on here. Very thin grafts of the Thiersch type are most easily cut from skin that has been rendered slightly turgid and hyperplastic by shaving three or four days beforehand, and this procedure was adopted alike for experimental grafts and controls in the great majority of tests. This preparatory treatment is objectionable only when mitotic counts in grafts of six days' standing are used to measure the degree of immunity (Medawar, 1946*a*).

To transplant a graft beneath the integument, a clean straight scalpel incision was made first through the superficial skin and then, avoiding any major skin vessel so exposed, through the panniculus carnosus. By inserting mosquito forceps through the incision horizontally (i.e. in the plane of the integument) and then slightly parting their tips, a tunnel was formed into which the graft could be tucked by a blunt probe, taking care only not to fold the graft upon its dermal surface. The initial incision was closed with a single suture clip.

The procedure adopted for transplantation to the brain was as follows: The full thickness of the skin was parted over a length of 5 mm. by a scalpel incision in the midline of the head following the parietal suture line. The skin—mobilized if necessary by cautious undermining—was then shifted in such a way as to carry the incision some 5 mm. towards the left side. The bony space underlying the incision in its new position was firmly scraped and then cautiously penetrated by a twist-drill 2 mm. in diameter. The membranes of the hemisphere being exposed, the graft was tucked into the bore of a standard tumour implantation trochar by folding it over on its cuticular surface and inserted vertically to a depth of 4–5 mm., whereupon the central plunger was thrust home. After withdrawal of the trochar the skin incision moved back to its original position medially and, after thickly dusting with sulphadiazine powder, was closed with two or three suture clips.

Transplantation to the anterior chamber proved to be a quick and easy operation: the cornea was penetrated at a point 2–3 mm. inwards of the limbus by a single stab with the tip of a very sharp ophthalmic scalpel. The graft was tucked through the slit so formed and manoeuvred into a central position by gentle strokes with a fine glass rod on the outside of the cornea. No special pains were taken to prevent the escape of the aqueous humour, which was fully regenerated in 24 hours.

Note on encystment.

Heterotopic skin grafts and cultures of skin in fluid media undergo or start to undergo encystment, the end-result being the formation of a closed vesicle.

Encystment may occur in one or other, or simultaneously in both, of two ways. The familiar dermal cyst of trapped skin epithelium is one in which the cuticular surface of the skin faces inwards and the basal layer faces outwards. Since the cuticle can be neither freed nor worn away, the characteristic appearance of such a cyst is an onion of cuticular layers bounded on the outside by a taut-looking layer of cells of the stratum germinativum. Such cysts, which are morphologically inside out, will be said to form "externally" (Fig. 7, 9, 13, 15). But in tissue cultures, and sometimes in brain and anterior chamber grafts, the

converse process happens: skin epithelium migrates over its own dermal substratum ("epiboly") and forms a cyst with cuticle outwards and basal layer inwards (Fig. 8, 11, 14). The histological examination of deep follicle epithelium and the thicker parts of the surface epithelium so entrapped shows that such grafts are often on the borderline of ischaemic degeneration when closure is complete. Cysts of this type will be said to form "internally." In this paper little turns upon the distinction between these two types of encystment, but it may prove to be of importance in interpreting the behaviour of homografts of cultivated endocrine gland tissue.

Grafts Transplanted beneath the Integument.

The experiments described in this section comprise six independent tests, each one making use of an independent pair of rabbits. Fifteen to 18 days after the grafting of the recipients with skin in high dosage from their respective donors, the immunizing grafts were found in all cases but one to have broken down. The recipients were then simultaneously regrafted with (*a*) a single control graft to a raw area on the skin of the chest, and (*b*) a graft of the same size, source and shape tucked beneath the panniculus carnosus. Examination *in situ* and autopsy were carried out six days later.

The control grafts showed almost complete survival in one recipient, various degrees of partial survival in three others, and total breakdown in the remaining two—a result unexpectedly faithful (in view of the small size of the sample) to the prediction based on the data of Medawar (1944), giving six days as the median survival time of "2nd-set" homografts.

All the grafts tucked beneath the integument were, or had earlier been, fully vascularized. Their apparent external encystment (Fig. 2, 4, 6) represents the outcome, not of active epithelial migration, but of the fact that the grafts were folded over on their cuticular faces before implantation. Apart from encystment, the six sub-integumentary grafts proved to be *identical* with their controls in the finest histological details: i.e. not only in the proportion and distribution of such epithelium as still survived, but also in the mode and degree of vascularization and vascular survival (the blood vessels in rabbit skin homografts being as a rule destroyed at the same time as the foreign epithelium) and in the pattern and intensity of the infiltration of the graft dermis by mesenchymal cells from the dermal vessels and graft bed. Three pairs of photographs illustrate this identity of response where the graft pairs show, respectively, full survival (Fig. 1, 2), partial survival (Fig. 3, 4), and total breakdown (Fig. 5, 6).

These experiments illustrate for the second time the general rule (Medawar, 1945) that the immune state called forth by grafting foreign homologous skin is systemic in compass, and not merely local: and, more particularly, they show that the survival of foreign epithelium is quantitatively uninfluenced by the presence or absence of native epithelium in the graft's neighbourhood. Cells of mesenchymal origin alone are present in sub-integumentary connective tissue.

Grafts Transplanted to the Anterior Chamber of the Eye.

The study of 48 single skin grafts transplanted to the anterior chamber makes possible the following general description of their crude morphology. Small

(1 mm. × 1 mm.) skin squares shift their position after implantation, and during the ten days allowed, as a standard procedure in all experiments, to pass before autopsy. At autopsy, the graft has been found either (*a*) weakly adherent to the corneal endothelium and free from other tissue and vascular attachments: or (*b*) attached more or less intimately to the pupillary border or the iris, or to its outer face in the angular space lying between the iris and the corneal endothelium: or (*c*) firmly imbedded in the anterior pole of the lens. Grafts ending in position (*a*) were never, and in position (*b*) were always vascularized. (On two occasions a graft attached to the corneal endothelium was connected to the iris by a delicate vascular pillar of "gathered up" iris tissue. These two grafts are, of course, classified under group (*b*).) Grafts found to be imbedded in the lens were unsatisfactory and showed ischaemic degeneration.

Whether vascularized or not, the epithelium of skin transplanted to the anterior chamber shows migratory and mitotic activity. A preliminary survey

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EXPLANATION OF PLATES.

FIG. 1–6.—Skin homografts six days after transplantation to a raw area on the chest (Fig. 1, 3, 5) and to a subintegumentary pocket (Fig. 2, 4, 6) on rabbits rendered immune by earlier graftings of skin from their respective donors. In the pair illustrated by Fig. 1, 2, the epidermis is almost wholly surviving and dermo-epidermal cleavage is no more than incipient. Fig. 3, 4, illustrate a low degree of partial survival, and Fig. 5 and 6, total breakdown. Encystment apart, the modes of response of the grafts in their two different positions are identical. All × 22.

FIG. 7.—A skin autograft which has become strongly adherent to and vascularized through the iris ten days after transplantation to the anterior chamber of the eye. Migratory activity of the epidermis has brought about complete external encystment, and sebaceous glands have formed anew. × 22.

FIG. 8.—As Fig. 7, but from a vascularized autograft seen in section at a level at which internal encystment is complete; the epidermis has abandoned its old cuticle and grown round upon its own dermis instead. × 22.

FIG. 9.—Contrast Fig. 7: a skin homograft ten days after transplantation to the eye of a specifically immunized recipient. The graft, intimately attached to the iris, has become vascularized and shows no trace of surviving epithelium. Note, however, that a high degree of external encystment had occurred before breakdown. × 22.

FIG. 10, 11.—A *non*-vascularized skin homograft ten days after transplantation to the anterior chamber of the eye of a specifically immunized rabbit (Fig. 11). The complete necrosis of the control graft on the chest (Fig. 10) is witness to the completeness of immunization. Although proliferation is subdued, migratory activity of the still fully-surviving epithelium has gone so far as to bring about an almost complete internal encystment. × 22.

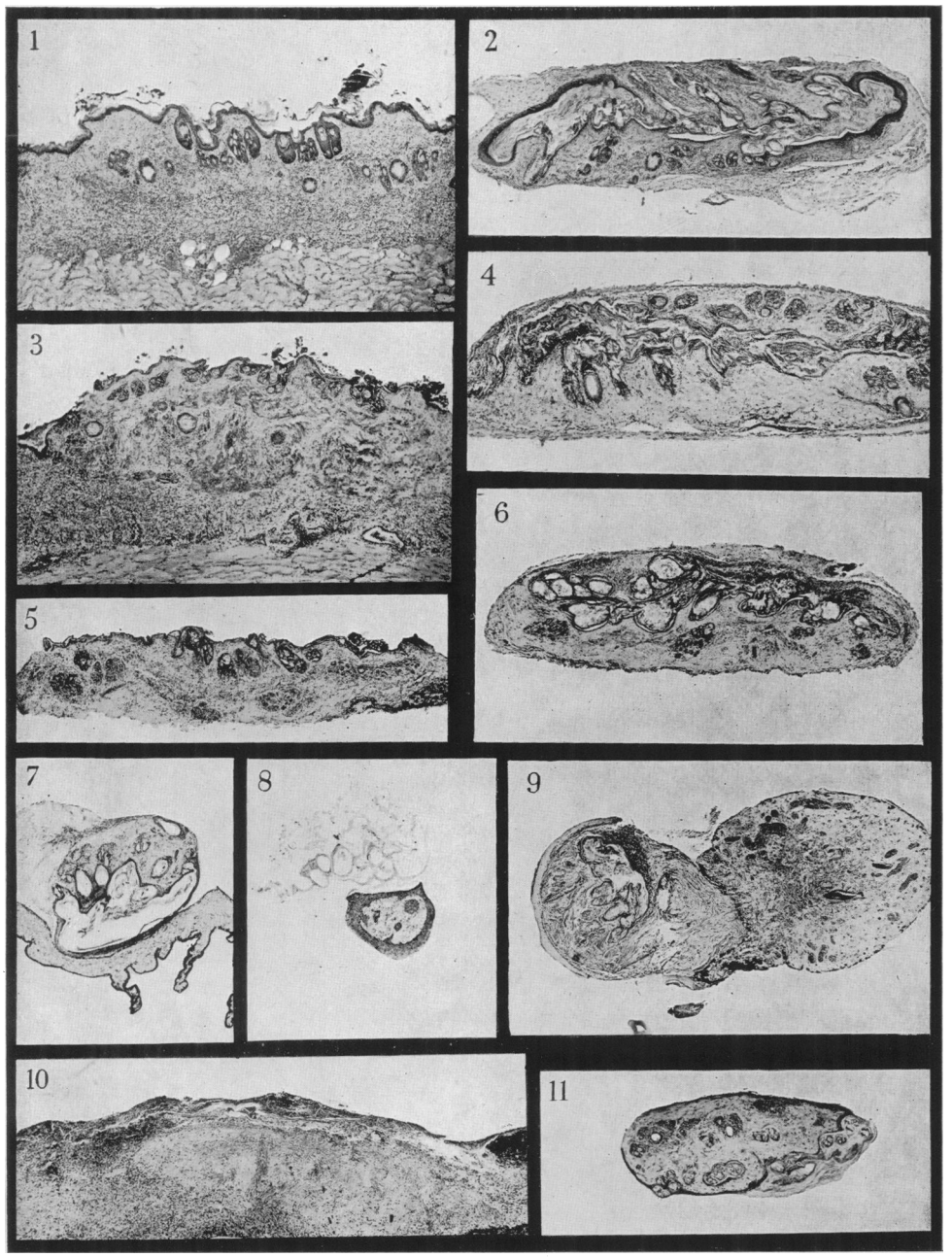
FIG. 12, 13.—As Fig. 10, 11, but from another pair of rabbits in which the surviving, non-vascularized homograft in the anterior chamber shows a mixture of internal and external encystment. × 22.

FIG. 14, 15.—Skin autografts ten days after transplantation to the brain. Note the vigorous epidermal proliferation, the rich population of dermal blood vessels, and the migratory activity of the epithelium, which has led to the beginnings of internal encystment (Fig. 14) or to a mixture of internal and external encystment (Fig. 15). × 22.

FIG. 16, 17.—Contrast Fig. 14, 15. Skin homografts ten days after transplantation to the brains of specifically immunized rabbits. Total breakdown of long standing. × 22.

FIG. 18, 19.—Illustrating the bare survival of a trace of epithelium at the margin of a skin homograft ten days after transplantation to the brain of a specifically immunized rabbit (Fig. 18): and the formation of isolated "cultures" of surviving skin epithelium in an almost wholly avascular region of the ground substance of the brain (Fig. 19); from a similar homograft which elsewhere has broken down completely. × 59.

All the photographs are of sections stained with Ehrlich's haematoxylin, aqueous orange-G, and alcoholic eosin.



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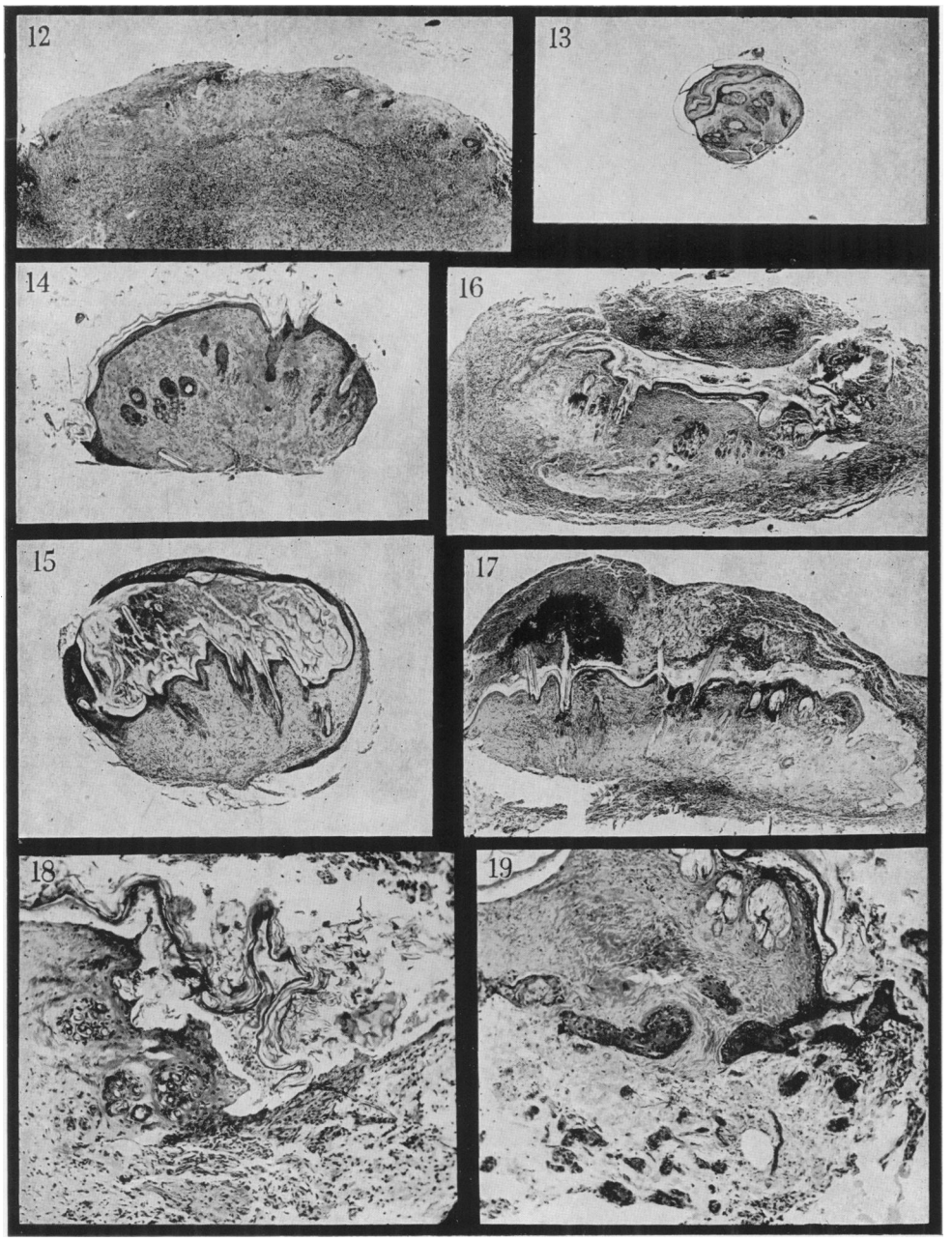

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of the growth of 10 independent autograft controls (Fig. 7, 8) showed that only three were vascularized after 10 days in residence. The two grafts with the greatest proliferation and migratory spread of epithelium—being also the only two to show new or persistent differentiation of sebaceous gland epithelium (Fig. 7) and the formation of hair follicle primordia—belonged to this group of three. Seven of the grafts showed full, and the remainder partial external encystment, accompanied in some cases by at least the beginnings of the migration of epidermis round dermis (Fig. 8). All the experimental grafts showed a similar preponderance of external encystment, and on this score there was nothing to choose between them.

Other autograph controls are mentioned in the report on the second experiment below.

First experiment.

This experiment comprises ten tests of the same form, each one making use of distinct but not independent donor-recipient pairs. (Two recipients had unique donors : the remainder shared four donors between them by pairs.) The initial immunizing grafts were found to have broken down completely when inspected 15–17 days after their transplantation, and the recipients were thereupon simultaneously regrafted with (*a*) a single control graft to a raw area on the skin of the chest, as before ; and (*b*) a 1 mm. × 1 mm. square in the anterior chamber of the right eye. Examination *in situ* and autopsy were carried out ten days later.

(It should be mentioned here that, by contrast with sub-integumentary grafts, there is one unavoidable source of disparity between such eye-implantation grafts as are vascularized and their invariably vascularized controls on the skin of the chest. The control grafts are penetrated by blood vessels within 48 hours. of planting, but eye implantation grafts may wander about free for several days before forming adhesions to the iris border or elsewhere. This delay of vascularization is sufficient to explain the fact that vascularized eye grafts may lag slightly but perceptibly behind their controls when undergoing breakdown.)

In all cases but one the control graft on the chest showed total breakdown of long standing after 10 days, in accordance with expectation (Fig. 10, 12). In the one exceptional case the recipient's original donor had died, and, rather than abandon the experiment entirely, the recipient was regrafted from a new donor source. The survival of the control graft 10 days after transplantation to its thus non-specifically immunized recipient provides yet another demonstration of the strong donor-specificity of the immune reaction (Medawar, 1946*a*). The anterior chamber graft in this animal showed full survival in spite of rich vascularization—an exception which proves the rule to be formulated below.

The remaining nine grafts fell into the following groups : (*a*) three fully vascularized grafts, of which two showed total breakdown (Fig. 9) and the third a barely perceptible fraction of epidermal epithelium which could still be judged surviving on histological evidence (Medawar, 1944, 1947) : (*b*) four grafts wholly devoid of vascular attachments which were in a fully surviving state. As with non-vascularized members of the set of 10 autograft controls, proliferation and migratory activity were subdued (Fig. 11, 13). Finally (*c*), two grafts which were found to be intimately and firmly adherent to the outer face of the iris, but

which failed to reveal the characteristic dilated and engorged capillary vessels on sectioning. Both showed partial survival of epithelium. It seems likely that these two represent an intermediate condition : late attachment to the iris and a correspondingly delayed and rudimentary penetration by iris vessels, with the consequence that breakdown, though in progress, was not complete at the time of inspection. A moderate leucocytic infiltration of these grafts showed that *some* effective vascular contact must have been established, despite the lack of overt evidence of the presence of blood vessels.

These experimental results show (as those to follow confirm) that *non*-vascularized eye implantation homografts are exempt from breakdown when transplanted to fully and specifically immunized rabbits. If allowance is made for the unavoidable disparities between their respective times of vascularization, they further show that eye implantation homografts penetrated by blood vessels, notwithstanding their minute size, participate fully in the specific degenerative changes undergone by their controls.

Second experiment.

It was thought worthwhile to repeat the preceding experiment as a whole with one modification : the single donor skin homograft tucked into the anterior chamber was accompanied by a graft of mesenchymal tissue from the recipient itself. Pairs of grafts so implanted form a composite ball of tissue which may or may not come to be vascularized.

The host's contribution to this compound graft was cut from the "active" (hyperaemic, turgid, rapidly proliferating) tissue of the bed of any one of the original immunizing homografts after their breakdown. The homograft was stripped from its bed and a cube of tissue 1–1½ mm. in length of side was snipped out from the middle of the vascular crater so exposed. One such cube was inserted with each donor skin homograft and gently manoeuvred to its side. The tissue of which it is composed is wholly mesenchymal, and consists of fibroblasts, histiocytes, miscellaneous leucocytes and vascular endothelium, supported by a delicate web of collagen fibres of new formation. (For a full description of the anatomy of the homograft bed, see Medawar (1944).)

In other respects, and with the added refinement that each recipient was independently paired with a single donor, the ten pairs of animals that comprise this experimental group were treated just like their predecessors. The recipients were regrafted from their respective donors 16–20 days after transplantation of the immunizing dose of grafts : and at autopsy ten days later the control grafts on the chest showed total breakdown of long standing in all cases. Immunization was thus demonstrably complete.

The standard double implantation test, repeated ten times, consisted of transplanting *D* skin + *R* graft bed tissue to one eye of *R* after its specific immunization by tissue from *D*. To control against the possibility of some non-specific toxic action by (e.g.) an exudate from the inflamed graft bed tissue, and to assess the magnitude of any specific contribution of its own to its neighbour's fate, six of the ten recipients were caused to receive *R* skin + *R* graft-bed tissue in the other eye. The donors corresponding to three of these six recipients received, in addition, *R* skin + *R* graft-bed tissue in one eye, and *D* skin + *R* graft-bed

tissue in the other. In the outcome, eleven of the twelve control grafts, five of them unvascularized, were wholly autograft-like and showed vigorous proliferative and migratory activity. The twelfth had become imbedded in the anterior pole of the lens and, like other such grafts, showed non-specific ischaemic changes. It may be concluded that any action attributable to the graft-bed component of the compound eye implantation graft is specific in character.

All ten experimental compound grafts gave evidence of migratory activity by the skin epithelium: predominantly, as before, of external encystment, but in some cases trivially complicated by the tendency of *D* epithelium to abandon its own cuticular investment and to grow round the *R* graft-bed tissue component. Fibroblastic cells in the *R* component survived and proliferated and showed some tendency to form a fibrous capsule round the dermal remains of such *D*-grafts as, having been vascularized, had broken down.

As with the first experiment, the ten compound grafts were found at autopsy to fall into three groups: (*a*) a group of six, totally broken down, of which five had heavy iris adhesions and were overtly vascularized, while the sixth had a fine iris attachment with only leucocytic infiltration to give evidence that vascular penetration had in fact occurred (see above). Two grafts, (*b*), were adherent to the corneal endothelium and unvascularized: these showed full epidermal survival; and (*c*) the remaining two showed partial survival and a weak marginal vascularization. As with group (*c*) of the preceding set of ten, these two are taken to represent grafts which had anchored themselves late to the iris and which lagged proportionately behind their controls in submitting to the immune reaction.

This second experiment therefore reinforces without enlarging upon the results of the first: grafts that are not vascularized are not appreciably affected by the recipient's specific immune state. When any appropriate allowance is made for inequalities of vascularization time, grafts that *are* vascularized submit to the immune reaction in the fashion of their orthotopic controls. The addition of mixed mesenchymal tissue from the bed of one of the recipient's broken-down homografts was without appreciable effect, at least in the dosage relationship of host to donor tissue here used.

Grafts Transplanted to the Brain.

In these experiments only one problem was at issue: whether homografts transplanted to the brains of specifically immunized rabbits survive, or submit to the immunity reaction and break down.

A series of six 10-day autograft controls showed brain tissue to be an admirable culture medium for grafted skin: the dermis of such grafts is richly vascularized, and this enables it to support a deeply stratified and strongly proliferating epithelium which encysts internally (Fig. 14) or externally or in both ways simultaneously (Fig. 15).

In the experimental series a graft was transplanted to the left cerebral hemisphere and simultaneously, as usual, to a raw field on the chest, 16–20 days after the preliminary specific immunization. Immunization was effective in all cases, and the orthoptic control graft on the chest became completely necrotic in the ten days allowed to pass between transplantation and removal at autopsy.

Four of six brain grafts so implanted likewise showed the characteristic

appearance of total breakdown of long standing (Fig. 16, 17). The cells of the graft epidermis, with pyknotic nuclei and strongly eosinophilic cytoplasm, had broken away from the dermis and in places were undergoing maceration. The dermis, destitute of any living element, was either clear or lightly peppered with twisted chromatic fragments representing the nuclear remains of leucocytes which had invaded the graft before, and during the earlier stages of, breakdown. Lymphocytes had aggregated in moderate density in the brain tissue surrounding the graft.

In the remaining two grafts breakdown fell just short of completion. In one (Fig. 18) a fragment of epithelium at the graft margin remained basiphilic in staining reaction, and there being some reason (however feeble) for supposing it to be alive, must be judged still living (Medawar, 1944, 1947). In the other (Fig. 19) the graft itself was necrotic, but two or three islands of epidermal epithelium in the tenuous and almost wholly avascular ground substance of the brain adjacent to it were still (by the same token) in a surviving state.

There is other evidence that the breakdown of grafts transplanted to the brains of immunized animals dallies slightly behind the breakdown of their orthotopic controls: for even in the four grafts found to be wholly necrotic at autopsy, the presence of a multilayered cuticle so disposed as to indicate the beginnings of encystment by internal or external closure showed that relatively vigorous mitotic and migratory activity had occurred before the immunity reaction finally overtook them. The cause of this time-lag is not clear. It should be noted, however, that this phase of proliferation and migratory activity overlaps the period during which the invading capillary sprouts may be supposed to have a temporarily increased and therefore abnormal permeability (Abell, 1946). Unless the presence of a skin graft permanently or for very long periods alters the characteristic permeability of brain capillaries in its neighbourhood (which is by no means impossible: Lange, 1944) it can be taken that the graft submits to the immunity reaction after a capillary circulation of not grossly abnormal permeability has been re-established.

DISCUSSION.

The role of vascularization in the homograft reaction.

In rabbit skin homografts the breakdown of dermal blood vessels is very nearly simultaneous with the breakdown of the foreign epithelium: and since some form of vascular stagnation or rupture accompanies the breakdown of skin and other tissue homografts in other common laboratory animals and in man, it has long been thought that the two events are related to each other as cause to effect (Fasiani, 1924).

There are many objections to this view, singly indecisive but together of some weight. For example, homograft epithelium which migrates from the centre of a pinch graft over richly and persistently vascular granulation tissue of native origin breaks down earlier than, if not simultaneously with, the "resident" foreign epithelium of the graft centre (Gibson and Medawar, 1943; Medawar, 1944): and where native and foreign epithelium are in contact, the breakdown reaction distinguishes the two types almost to a cellular boundary (Medawar 1944). It is hard to believe that a process of vascular breakdown could discrimi-

nate between the two so finely. In feebly immunized animals, moreover, the progress of the immune response is indicated by the partial suppression of hyperplasia before the blood vessels of the graft dermis show any perceptible specific abnormality (Medawar, 1946*b*).

The behaviour of homografts transplanted to the anterior chamber of the eyes of specifically immunized rabbits now provides an almost decisive disproof of the hypothesis that ischaemia is the immediate cause of homograft breakdown. For if such grafts are vascularized they are destroyed, and their blood supply is duly arrested: but they do not need to be vascularized in order to remain alive. If the interruption of blood supply were the cause of breakdown, then such a graft should remain alive as a sort of tissue culture *in vivo*, being now on all fours with those eye-implantation homografts that are, in fact, never vascularized at all. They do, however, succumb completely. Vascular breakdown is not, therefore, the primary mode of expression of the immune reaction.

The aqueous humour is variously regarded as a plasma ultrafiltrate or as an active secretory product. In either event it is normally free from protein. The survival of non-vascularized skin homografts in the eye, despite adhesion to the corneal endothelium or very intimate association with proliferating mesenchymal tissue of native origin (see above), and their prompt breakdown after vascularization, suggests that the immune reaction is mediated by blood plasma or by cells transported in it. The systemic nature of the immune reaction (further affirmed by the experiments described in this paper) is consistent with no other belief.

The role of lymphatics.

Homografts transplanted to the brain and (according to one view: see above) the anterior chamber of the eye do not elicit an immune reaction. Homografts transplanted to the brain or which become vascularized in the anterior chambers of animals already specifically immunized do, however, submit to it normally. This disparity of behaviour is consistent with the view that a lymphatic drainage system is required to create a state of immunity but not necessary to enforce a response to it. A similar disparity was recorded by Medawar (1946*b*) in assessing the immunizing power of leucocytes towards skin subsequently grafted from the leucocyte donor. Leucocytes are at least 18 times more effective when injected intradermally than when injected intravenously, i.e. at the point physiologically most remote from lymph nodes in general and from any one set of draining nodes in particular.

Clinical significance: the corneal homograft.

It is now a commonplace of ophthalmic surgical practice that a corneal defect may be repaired by a graft of corneal tissue taken from someone other than its intended recipient. There are at least four possible reasons for the success, or apparent success, of such corneal homografts:

(1) The dosage of foreign tissue involved in corneal homografting may be ineffectively small (Medawar, 1944, 1945). There may indeed (though it has not been demonstrated) be a threshold of dosage below which a homograft cannot immunize its recipient strongly enough to secure its own breakdown. McDonald and Medawar, in unpublished observations, find that mouse skin homografts

differing only slightly in antigenic equipment from their recipients may struggle along and survive despite a manifestly feeble immunization.

(2) Because of the feebleness of immunization, breakdown (if it occurs) may be delayed in onset and prolonged in execution. (Breakdown of the cell population of a rabbit skin homograft may take up to four days from beginning to end; Medawar, 1944). Since corneal epithelium is highly mobile (Arey and Covode, 1943; Mann, 1944), cells of native origin from the periphery of the grafted area may insidiously and imperceptibly replace the foreign epithelium during its protracted breakdown.

(3) Corneal homografts cannot elicit immunity, even if they could succumb to it.

(4) Corneal homografts, being unvascularized, cannot succumb to an immune reaction even if they can initiate one.

The experiments reported on here show that explanation (4) is singly sufficient. If one of the two controversial views outlined in the introduction is accepted, explanation (3) is likewise singly sufficient. If neither (3) nor (4) were true, nothing would prejudice the applicability of explanations (1) and (2) So far from being in any way anomalous, therefore, the observed clinical success of corneal homografts has a stronger theoretical backing than most surgical procedures aspire to.

SUMMARY.

1. Heterotopic grafting has been used as a means for studying the part played by blood and lymph vessels in transplantation immunity.

2. The breakdown of skin homografts transplanted beneath the integument of specifically immunized rabbits is indistinguishable in degree and kind from the breakdown of control grafts transplanted ("orthotopically") to the skin of the chest.

3. A skin homograft transplanted to the anterior chamber of the eye of a specifically immunized rabbit is destroyed if, and only if, it is penetrated by blood vessels.

4. The fact that vascularized eye implants are destroyed, though they are not dependent on a blood supply for their continued survival, shows that vascular stagnation is not causally connected with the breakdown of skin homografts.

5. Skin homografts transplanted to the brains of specifically immunized rabbits respond by total breakdown to an immune state already in being. On the testimony of other workers, they enjoy prolonged or indefinite survival when grafted to the brains of non-immunized animals.

6. It is concluded that skin homografts transplanted to the brain submit to but cannot elicit an immune state.

7. It is suggested that the presence of a lymphatic drainage system is necessary for immunity to be called into being and that penetration by blood vessels must occur before it can come into effect.

8. The behaviour of heterotopic grafts of all kinds shows that the immune state provoked by skin homografting is systemic in compass and not confined to a reaction in the neighbourhood of the foreign grafts.

9. The clinical success of corneal homografts is explained.

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THE EPIZOOTIC BEHAVIOUR OF MOUSE-POX (INFECTIOUS ECTROMELIA).

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INFECTIOUS ectromelia was first described by Marchal (1930) as a natural virus disease of mice. Following an observation that ectromelia virus produced a haemagglutinin which agglutinated the same restricted group of erythrocytes as did vaccinia virus, Burnet (Burnet, 1945*a*; Burnet and Boake, 1946) established the close immunological relationship between ectromelia and vaccinia, and pointed out that ectromelia was the murine representative of the mammalian pox viruses. Consideration of the symptomatology and epidemiology of the disease led to the suggestion by Professor Burnet (Fenner, 1947*c*) that the disease should be called mouse-pox. I have adopted this name as a synonym for ectromelia when discussing the actual disease in experimental mice, but have retained "ectromelia" to designate the virus itself.

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